Microbial Source Tracking (MST) Analyses of three Connecticut Watersheds of Long Island Sound.

Project #13-04e: A project funded by the CT-DEEP through a CWA Section 319 Grant

NEHA AEC 2019 – presented by **David Knauf & Michael Pascucilla**

Long Island Sound

Once called the "American Mediterranean" and more recently described as "The Urban Sea," Long Island Sound is considered one of North America's most urban yet biologically diverse estuaries.

Long Island Sound (LIS) has been designated as an Atlantic Ocean tidal estuary of national significance by the United States Congress. With a population of over nine (9) million people living within the watershed area, its coastline is shared by three states.

Long Island Sound is 21 miles (34 km) at its widest point and varies in depth from 65 to 230 feet (20 to 70 m). The Sound, which is a mix of freshwater from tributaries and saltwater from the ocean, generates between $17 billion to $36.6 billion in economic value every year from such activities as commercial and recreational fishing, shellfishing, seaweed farming, boating, tourism and other commercial & recreational activities.

However, due to public health concerns, swimming waters and shellfish beds are closed when bacteria levels are elevated, causing residents and commercial users to lose access to these resources. But what are the sources of bacteria? Human sewage? Agriculture? Birds and wildlife? And what is the risk to public health?


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NO PESCAR LOS MARISCOS TAL:
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CONNECTICUT DEPARTMENT OF AGRICULTURE
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Microbial Source Tracking (MST) Analyses: The Study

Three Connecticut local health departments, the Darien Health Department, the East Shore District Health Department and the Westport-Weston Health District, collaborated to submit a grant application to CT DEEP for conducting a MST of three distinct LIS watersheds known to have impaired water quality. The intent of the study was to use MST to determine the most likely sources of bacteria so remedial actions could be taken to reduce the bacteria levels. The application was approved by DEEP in September 2015 and the subsequent study involved laboratories at Yale University, the Connecticut Agricultural Experiment Station, and Earthplace. Water samples were collected by Health Department staff in each watershed monthly from January to December 2016.

Yale

PROJECT TEAM

Mark A.R. Cooper	**Project Manager**	**Westport Weston Health District**
Adalgisa Caccone	**Project QA Officer**	**Dept. Of Ecology and Evolutionary Biology, Yale University,**
Douglas Dingman	**Project QA Officer**	**Connecticut Agricultural Experiment Station**
Peter Fraboni	**Project QA Officer**	**Associate Director Harbor Watch Program Microbial Laboratory**
Lauren Brooks	**Laboratory Tech**	**PhD.**
Michael Pascucilla	**Director of Health**	**East Shore HD Lower Farms River and Goodwives Watershed**
David Knauf	**Director of Health**	**Town of Darien, Good Wives River Watershed**

Steve Dimatte, USEPA Project Officer

Christopher Belluca, CT-DEEP Quality Assurance Officer

Microbial Source Tracking (MST) Analyses in Long Island Sound Watersheds

- The three watersheds evaluated were: Sasco Brook in Westport, the Lower Farm River Watershed in Branford, and the Goodwives River in Darien, all in Connecticut.
- Each has a history of elevated bacteria counts, especially after rainfall events, leading to the closures of local beaches for contact recreation (swimming) and/or shellfishing. The public health risk and sources of bacteria were unknown.
- In addition, kelp farming and other fisheries being developed in LIS could be impacted by elevated bacteria counts, resulting in lost economic opportunities for Connecticut.

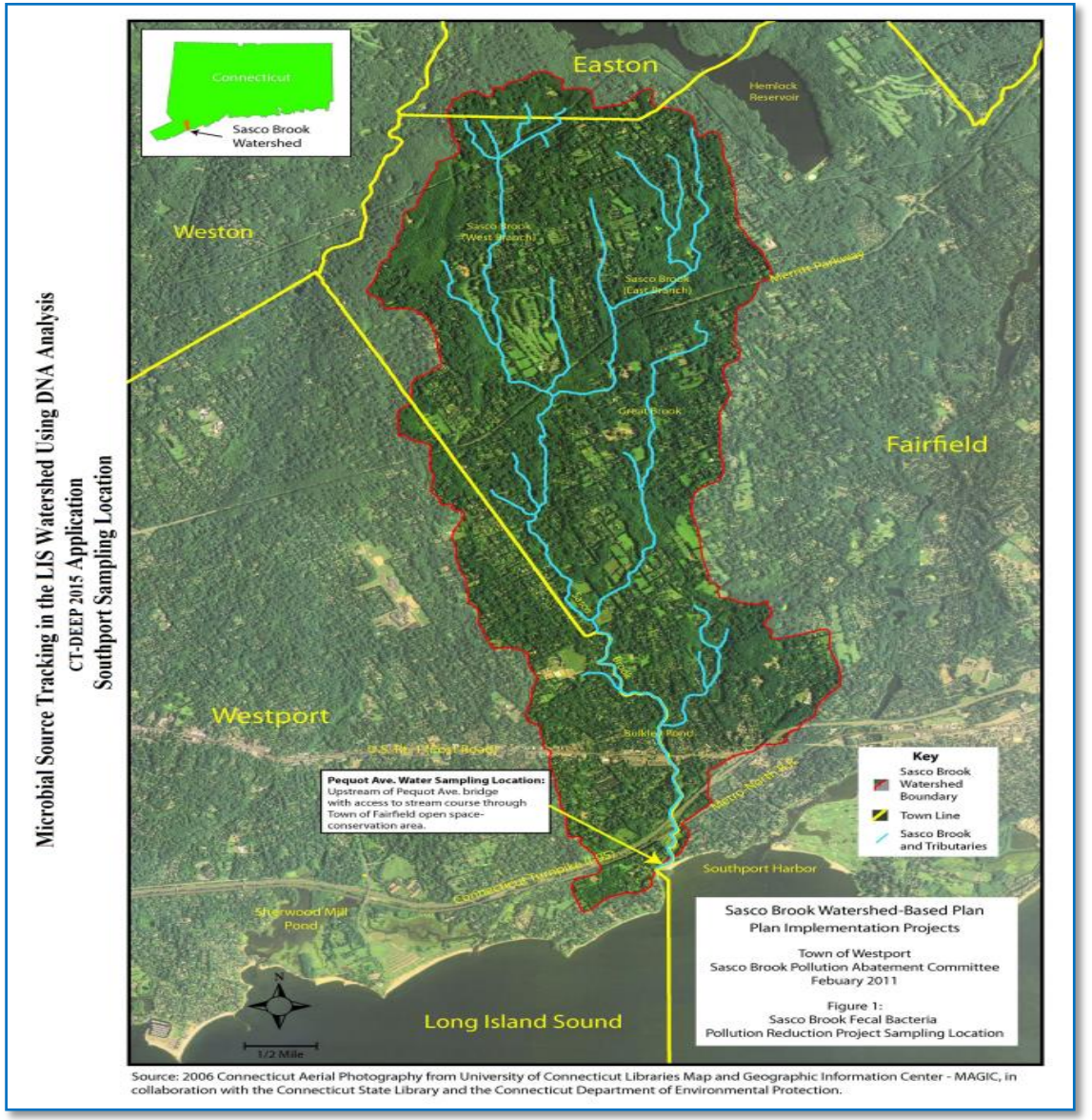
Westport Weston Health District

Microbial Source Tracking (MST) Analyses in the ***Sasco Brook***

Project Lead: Mark Cooper, Director of Health

Watershed Area: Saco Brook, Westbrook, Connecticut

Description: The Sasco Brook Watershed is located in southwestern Connecticut, encompassing portions of the Towns of Fairfield, Westport, and a small area of Easton, Connecticut. The brook drains a watershed of 10.2 square miles from its headwaters near the Easton/Fairfield. Sasco Brook has been identified by the CT DEEP as an impaired water body not meeting state water quality standards due to periodic elevated bacteria levels. The Sasco Brook Pollution Abatement Committee (SBPAC), a voluntary alliance consisting of representatives of local, state, and federal agencies, private organizations, and interested citizens, was organized to identify sources of bacterial contamination and pursue initiatives to improve water quality. Several SBPAC initiatives have resulted in the successful reduction of the detected amounts of bacterial contamination in Sasco Brook. But some bacteria, from unknown sources, is occasionally detected.



Learning Objectives and Goals:

Westport Weston Health District

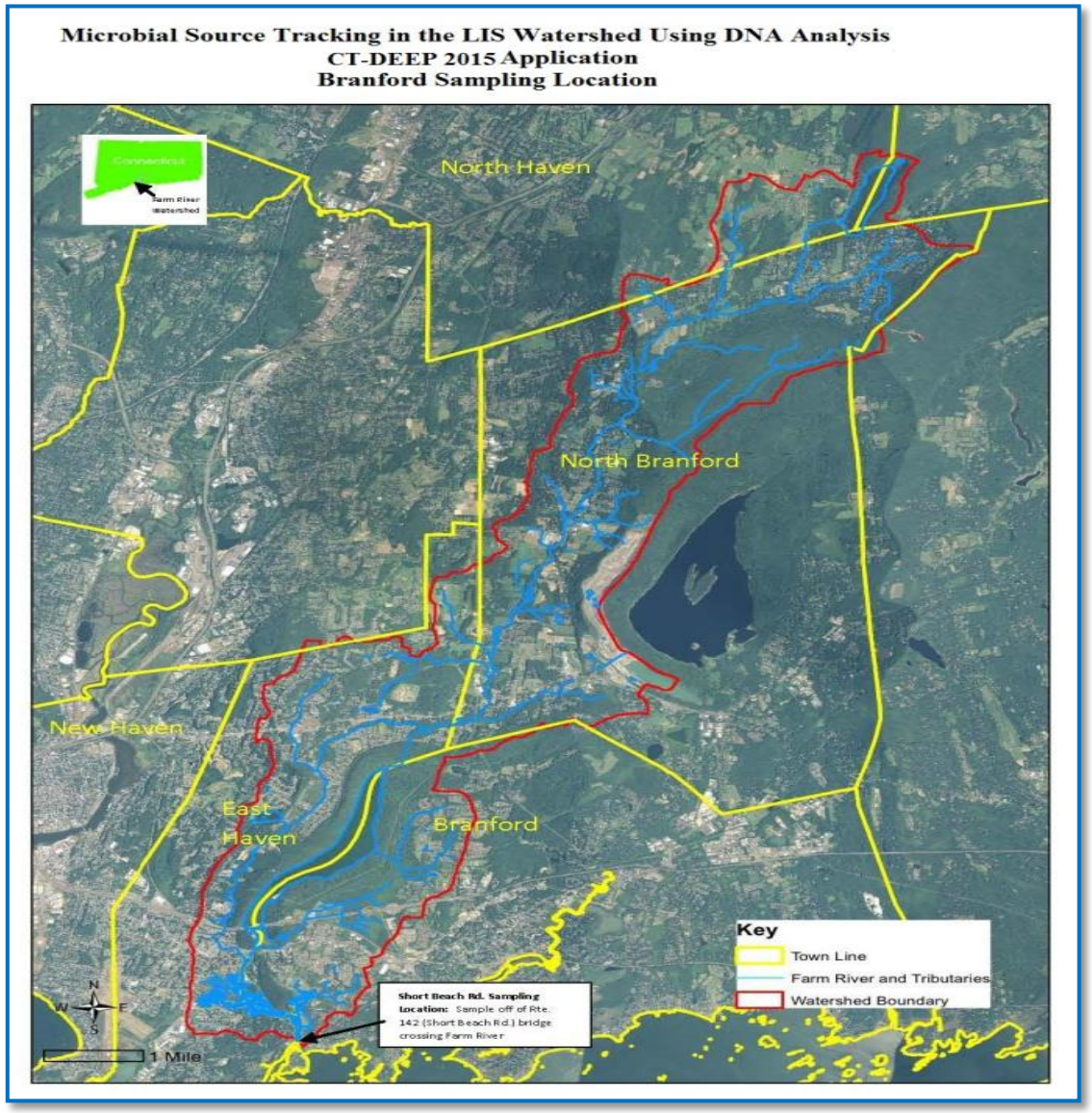
- Validate or refute the findings of the assumption-based methodology presumed in the TMDL (Total Maximum Daily Load) developed for use in the **Sasco Brook Watershed Based Plan**.
- Define a microbial source tracking program and test methodologies that could be implemented in future source tracking studies.
- Monitor the water quality within each subject watershed to more accurately identify significant human and non-human sources of fecal bacterial contamination to develop management strategies for reducing and/or eliminating identified bacteria sources.

Microbial Source Tracking (MST) Analyses in the ***Lower Farm River***

Project Lead: Michael A. Pascucilla, Director of Health

Watershed Area: Lower Farm River, Branford, Connecticut

Description: The Farm River Estuary is a wild, pristine and enormously complex productive, and fragile estuarine ecosystem with wooded uplands, high quality fresh water, and brackish tidal marshes. These picturesque floodplains, coves, and inlets provide a critical natural habitat for an immense variety of microbes, plants, insects, amphibians, reptiles, birds, fish, shellfish and mammals that rely on it for food, water, and shelter, especially during migration and breeding. The Farm River drains parts of Durham, Wallingford and North Branford on its 16.5 mile journey past Farm River State Park to Long Island Sound. The Farm River has been used for navigation by a fertilizer factory, stone quarry, paper mill, saloon, salt hay harvesters, fisherman and boating enthusiasts.



Learning Objectives and Goals:

- Understand the current State and EPA water sampling protocols.
- Learn the importance of local health department interventions of improving water quality within our communities.
- Recognize the importance of DNA sampling as a public health water quality tool.

Background/History

- In 2011 - 2012, our local health department teamed-up with Yale University to improve our local water quality.
- Working with a Graduate-level Student, we embarked on a non-approved EPA source tracking research study.
- Goal was to determine whether the bacteria in water samples was from human or animal sources.
- Results indicated it was mostly animal, non-human contamination.

Background Continued:
Bacterial contamination in Long Island Sound: improving beach closure policy and assessing the effects of climate change

- In 2016, we worked with a team of Yale students to investigate better marine beach water closure policies – Three (3) Aims:
  - **Aim 1:** Based on retrospective data, to examine the relationship between weather-related and other variables and bacterial contamination in Long Island Sound bathing water
  - **Aim 2:** Potential predictors will include rainfall amount, air temperature, water temperature, humidity, and tide. The endpoint will be enterococcal organisms per 100 ml. Effect modification by beach and temporal trends in predictors will also be examined.
  - **Aim 3:** To perform a critical review of saltwater beach closing policies in Connecticut and nationally, examining pros and cons of various policy options and making a policy recommendation for Connecticut local beaches. The report included an assessment of the effect of climate change on bacterial contamination and beach closure policy.

Background Continued:
Bacterial contamination in Long Island Sound: improving beach closure policy and assessing the effects of climate change

- Finding: Current policy did not effectively protect the public health of our residents/visitors.

- Specifically, prior to closure, two samples (initial and resample) were needed, exceeding a concentration of enterococcal organisms greater than 104 CFU/ 100 ml.

- Thus, there was a 24-48 hour window of potential unnecessary exposure to high bacteria levels for recreational marine water activities.

- Improvements were necessary to our beach closure policy, to include preemptive beach closure if rainfall exceeds a certain threshold.

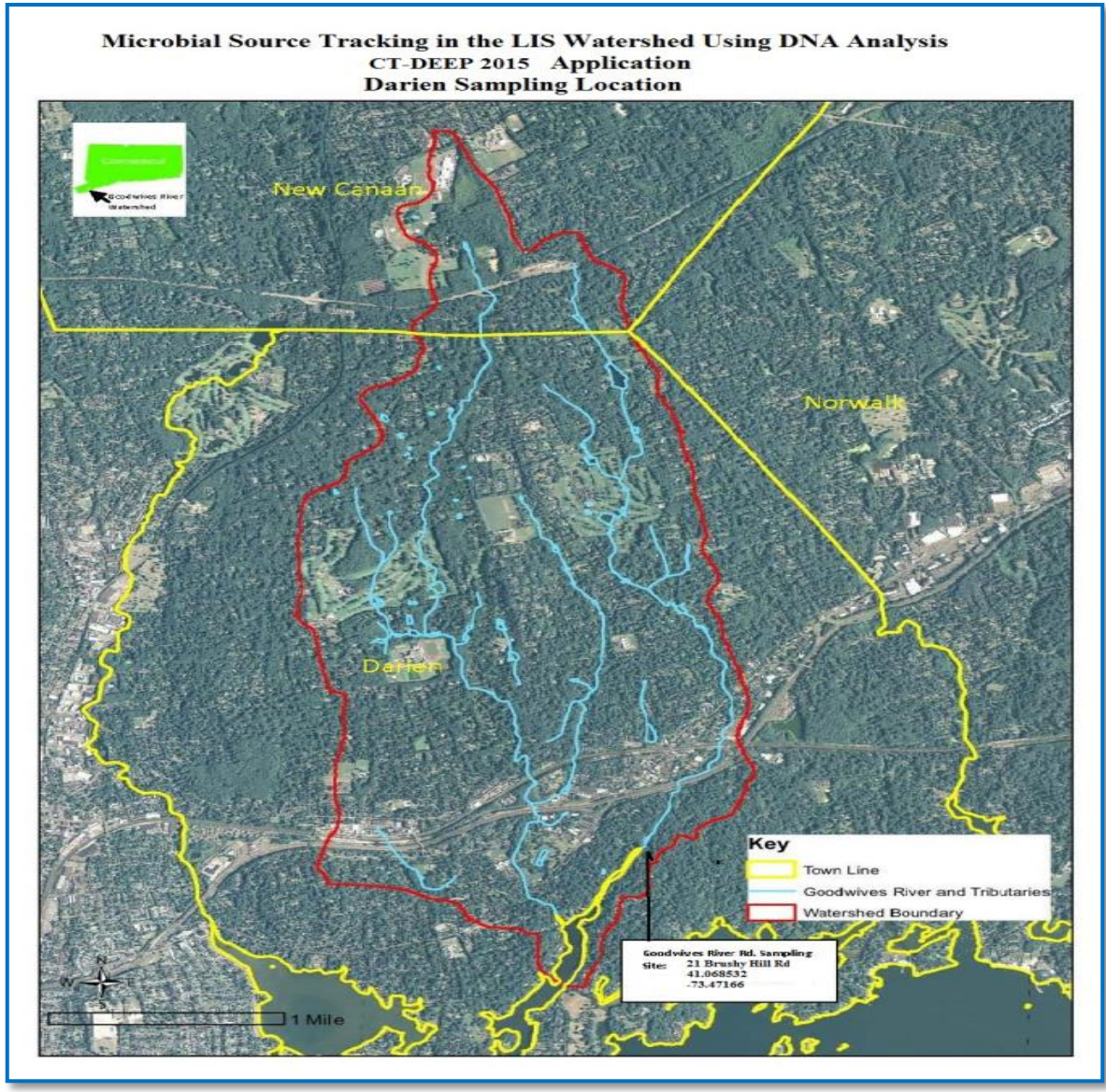
- Based on water sampling data of 9-13 years (depending on the Town), we established a 2.0 inch preemptive beach closure policy (Within a 24-hour Period)

Microbial Source Tracking (MST) Analyses in the ***Goodwives River*** Watershed.

Project Lead: David Knauf Director of Health

Watershed Area: Goodwives River, Darien, Connecticut

Description: Like Sasco Brook, Darien Cove has also been classified as impaired by CT DEEP for not meeting state water quality standards regarding fecal coliform (a class of organisms including *E. coli)* levels. This classification has prevented the harvesting of shellfish for direct consumption in this area. Additionally, water samples collected by the Darien Health Department at various locations along the Goodwives River, the primary source of fresh water to the Darien Cove, have shown consistently elevated levels of bacteria since 2009. According to the assessment conducted by the CT DEEP, likely sources of the bacteria are storm water and non-point sources, including decentralized treatment systems (such as septic systems), vessel discharges, and waterfowl. Detailed sanitary surveys have been conducted throughout the watershed with numerous potential but inconclusive sources of bacteria found.



As previously noted, the US EPA has established a standard of 104 enterococci per 100 ml of water as an indicator organism of possible contamination which could pose a risk to public health.

- Prior to 2008, Darien had a protocol that included beach closures if a single sample exceeded the EPA limit, as well as requiring closure if rainfall exceeded 1 inch within a 24 hour period.

- During the 2009 and 2010 summer seasons, a summer intern was utilized to perform water testing under a variety of conditions – high tide, low tide; before, during and after rainfall events; and at variable depths. We found bacteria levels remained relatively constant regardless of the tide cycle, but varied considerably during and after rainfall events, and returned to acceptable levels with respect to bacteria after 24 hours, which is two tide changes.

- The beach closure protocol was modified to eliminate closure due to a single bacteria exceedance, but the one inch rainfall trigger remained in effect.

- Water samples were taken at various locations along the Goodwives River which is the freshwater source closest to the beach with the most variable water quality. Elevated bacteria levels were consistently found, but without any obvious sources or discharges.

- So the question remained: *"**where are the bacteria coming from**?"*

RESULTS & DISCUSSION

- Established DNA markers for feces of poultry, dogs, cattle, humans, ruminants (sheep and deer) and birds were utilized.
- However, two assays specific for seagulls and birds failed to pass the laboratory screening and could not be included in further analyses. Thus, the study could not validate nor refute the significance of geese or seagulls as a source of pollution, even though they were periodically observed at collection sites. Also, no markers were available for rodents, thus the contribution to bacteria levels from rodents could not be quantified.
- *Significant* evidence of human or pet contribution to the bacteria was not found in any of the sampling locations.
- Traditional monitoring for fecal contamination relies on the culturing of fecal indicator bacteria (FIB). Numerous short comings associated with these methods have since been identified, including a lack of correlation with pathogen counts or reported illnesses (Colford et al., 2007; Wade, Pai, Eisenberg, & Colford, 2003), and the fact that samples must be cultured for 24 hours before results are known.
- Bacteria found at a beach or shellfish beds may originate in beach sand or be transported downstream, making it difficult to pinpoint specific non-point sources.
    - ***The bottom line: test results were unable to determine the source of bacteria.***

Future Considerations

- Limitations posed by using indicator organisms, such as enterococcus, illustrate the need to develop other methodologies for determining sources of potential contamination and assessing the risk to public health.
- An additional or alternate direction for future studies could be to employ next generation sequencing technologies to assess likely sources of bacteria, and to attempt to detect actual pathogens rather than focusing on surrogate indicators.
- Better, more reliable DNA marker assays are needed.
- Additionally, a flexible sampling schedule could be utilized including sampling during a rainstorm.
- This approach could also be used to evaluate whether the discrepancies between E. coli and GenBac results are due to the differential survival rates in the environment.

True Causes of Water Quality Deterioration

- Human waste is only one cause, and in many cases is not the main source.

- Human related activates:
  - Farming & Agriculture
  - Marine Waste
  - Industrial
  - Residential

- Natural Occurring:
  - Wildlife

How Harmful Is Bacteria When it is Present?

- Surface water is not sterile.
- Fresh Water vs Marine Water – They are very different.
- Presence of bacteria does not mean it is harmful/pathogenic.
- Not all bacteria present a risk to human health
- Age of the bacteria present in surface water appears to matter – a lot.

Next Steps

- Public Health Officials need to go beyond the reoccurring cycle of closing and opening bathing areas, beaches, shellfish beds and other recreational activities, especially when the time between sampling and results is so significant.

- Todays technology allows us to get to the root cause of water quality fluctuation and/or deterioration.

- Environmental Laboratories should consider expanding services to provide a tool to provide instantaneous bacteriological results and MST testing.

- EPA Approved Tests for MST for both human and non-human markers are needed.

- Process of submitting this water quality research to the NEHA Journal to continue the conversation.

Our Local Public Health Commitment

Change starts locally and we are doing our part to improve water quality.

Questions/Comments